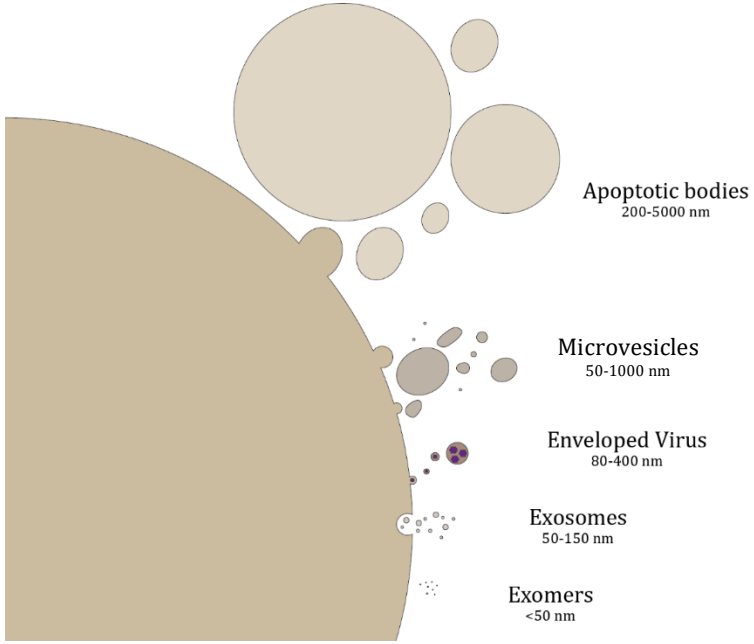
Philipps Universität Marburg

Using single-cell sequencing and Cre-lox reporters for tracking tumor EVs

Dr. Christian Preußer
EV Core Facility
Institute for Tumor Immunology
Center for Tumor Biology and Immunology (ZTI)
christian.preusser@staff.uni-marburg.de

Extracellular Vesicles

- Extracellular vesicles (EVs) are delimited by lipid-bilayer particles that can contain various components from their originating cells
- Key component of the cellular secretome



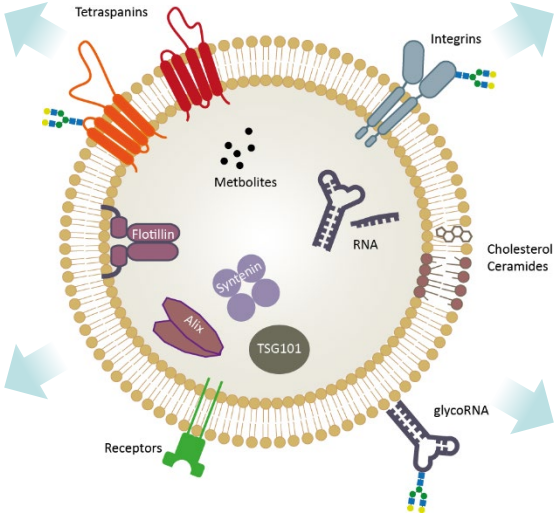
Regulation of transcription and translation

Survival and proliferation

Reproduction and development

Host-microbiome interaction

Disposal



Balance of immune response

Receptor-ligand signaling

Apoptosis

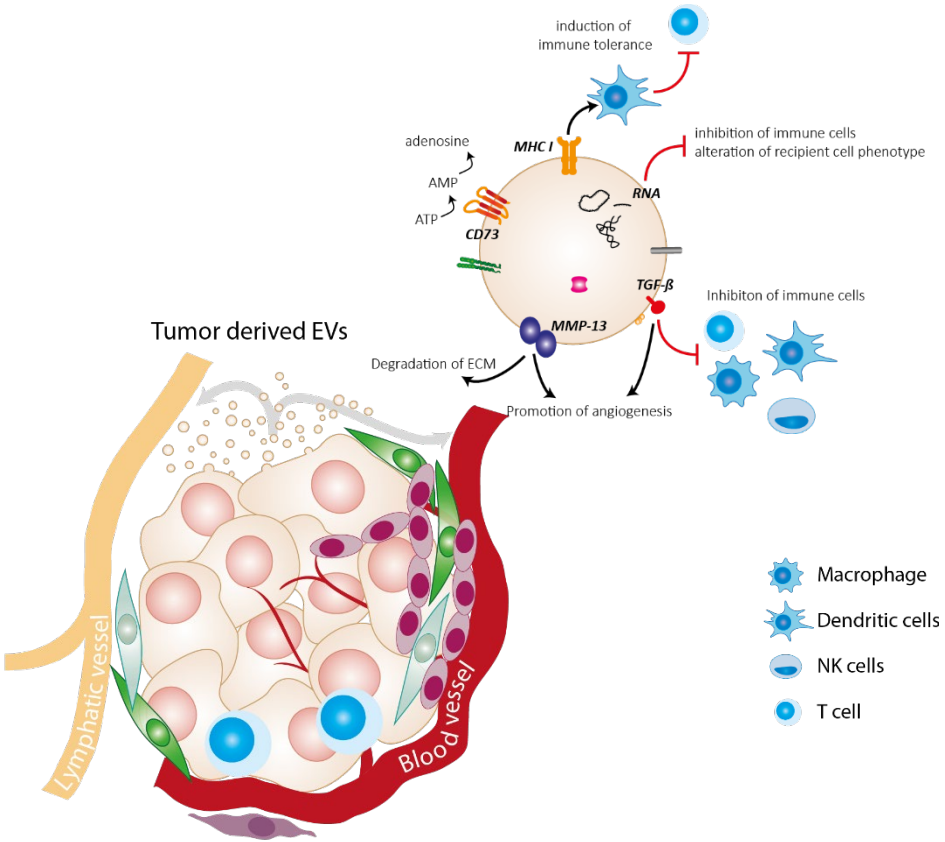
Cellular Differentiation

Cellular migration

Extracellular Vesicles in the Tumor Microenvironment

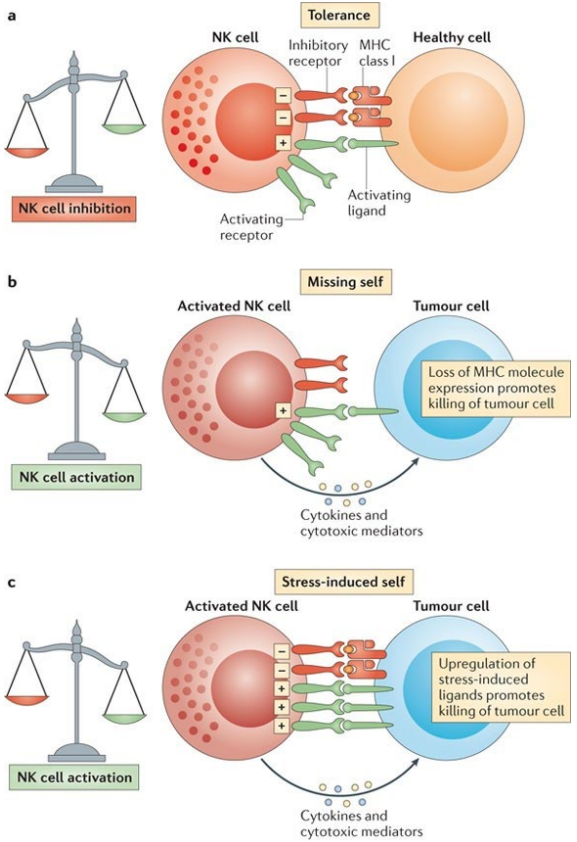
Diverse actions of EVs within the tumor microenvironment

- Enhancing angiogenesis
- Activation of stromal cells (normal fibroblast -> cancer associated fibroblasts)
- ECM remodeling
- Metastasis
- Therapy Resistance
- **Immunoediting (activation/inhibition of immune cells)**



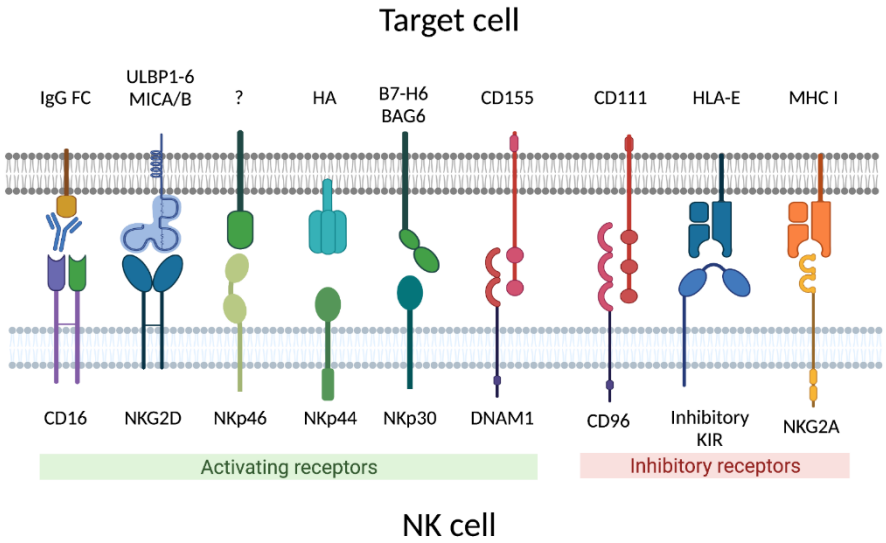
Natural Killer (NK) cell receptors and ligands

- 10-20% peripheral blood lymphocytes
- Can kill indiscriminately
- Cytotoxic cells (perforin, granzyme)
- Killing of tumor/infected cells
- Activated when stress ligands and/or **no** MHC I on target
- Depends on two types of receptors
  - Activating / Inhibitory
  - In normal cells, inhibitory receptors override activating signals



Vivier et al. 2012

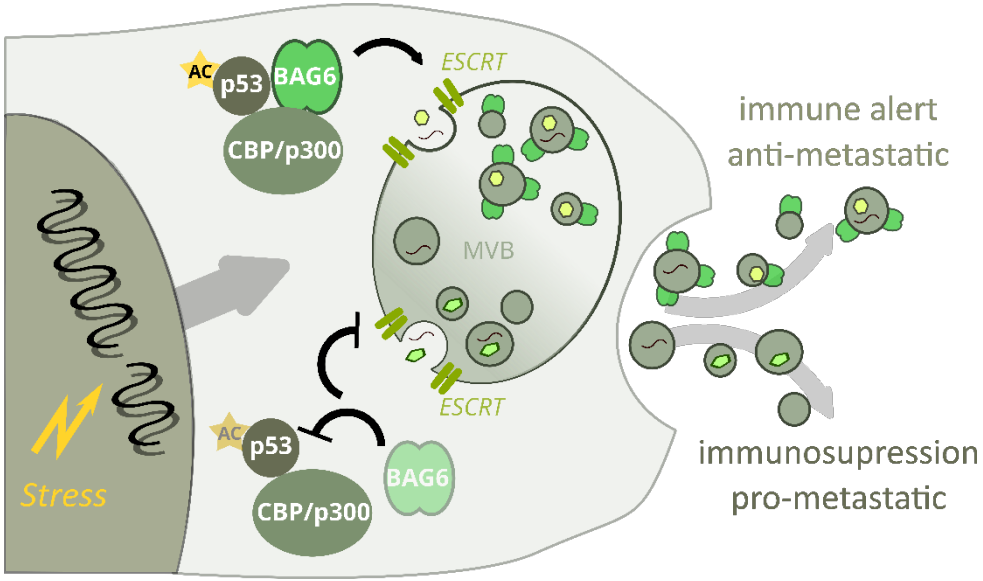
Natural Killer (NK) cell receptors and ligands



- Natural Cytotoxicity Receptors (NKp30, NKp44, NKp46)
  - B7-H6, **BAG6**, viral HA, pp65, etc.

    Bcl2-associated anthanogene 6

Natural Killer (NK) cell receptors and ligands
BAG6



- Natural Cytotoxicity Receptors (NKp30, NKp44, NKp46)
  - B7-H6, **BAG6**, viral HA, pp65, etc.

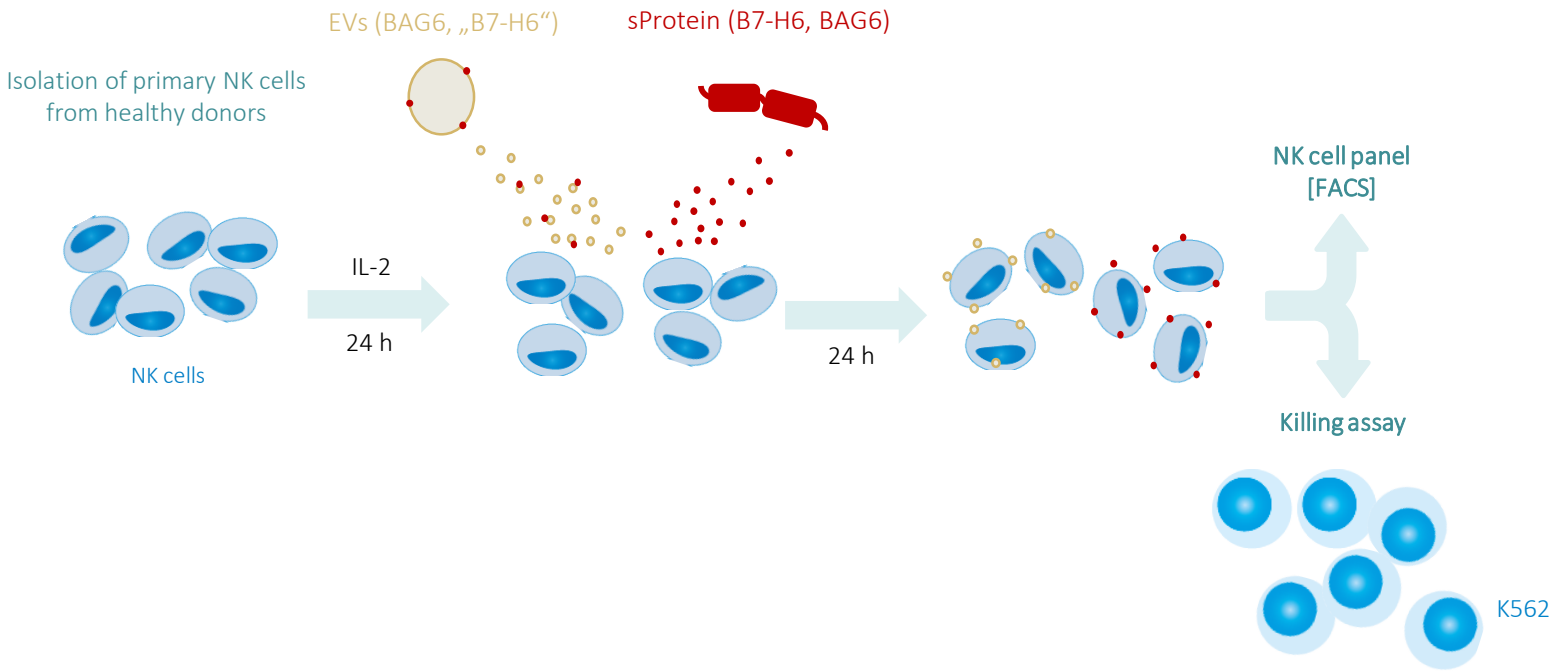
    Bcl2-associated anthanogene 6

Membrane and extracellular function:

- **regulates NK cell activity.** Pogge von Strandmann et al. 2007, Simhadri et al. 2008, Reiners et al. 2013
- **promotes T cell responses.** Rangachari et al. 2012
- **Inhibition of pro-metastatic neutrophils** and increased accumulation of anti-tumor patrolling monocytes. Schuldner et al, 2019

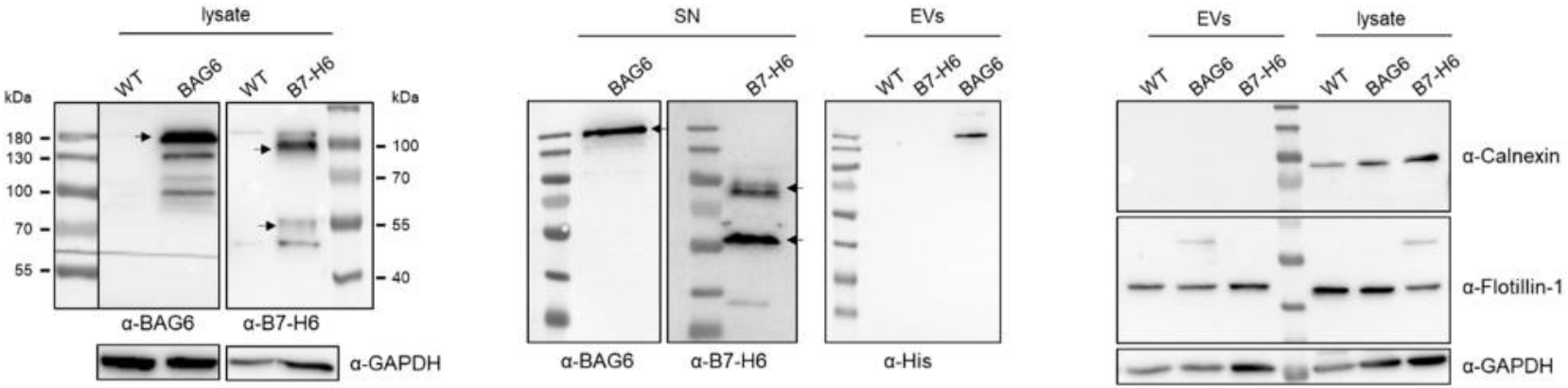
Elucidating the nature of NKp30 ligands and their impact on NK cell activity

In vitro set-up for analyzing different ligand formats



K562: myelogenous leukemia line; lack MHC complex

Overexpression of BAG6 & B7-H6 in HEK293 cells

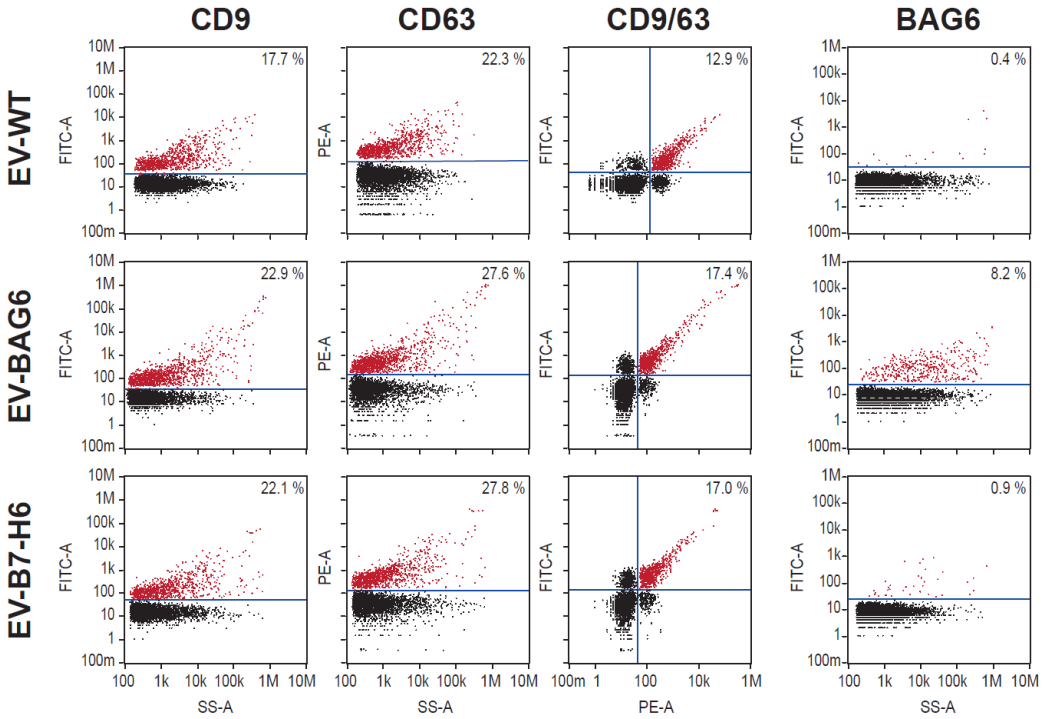


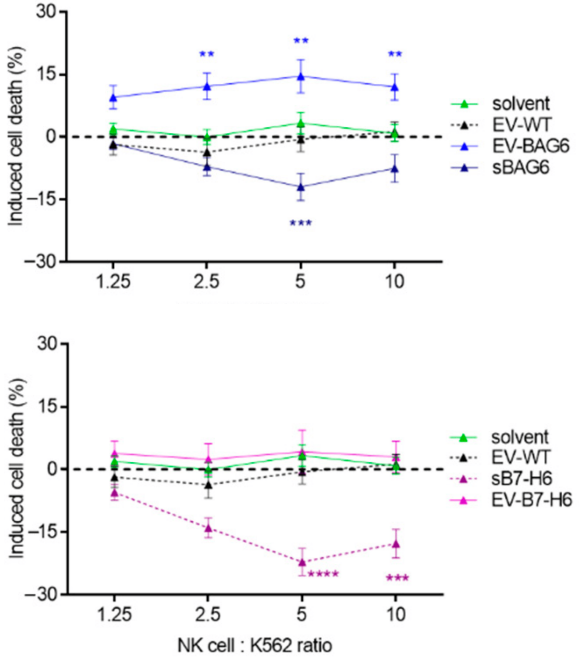
Western blot

- Protein present in the supernatant (after 100k xg)
- BAG6 associated with EVs, B7-H6 **not**

Ponath et al. 2021

Soluble BAG6 protein diminish NK cell killing response





Ponath et al. 2021

Nano-flow cytometry

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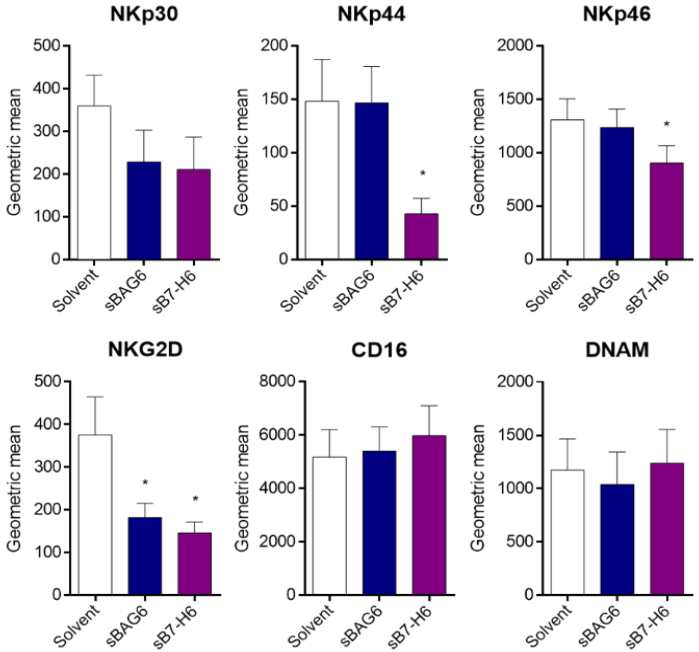
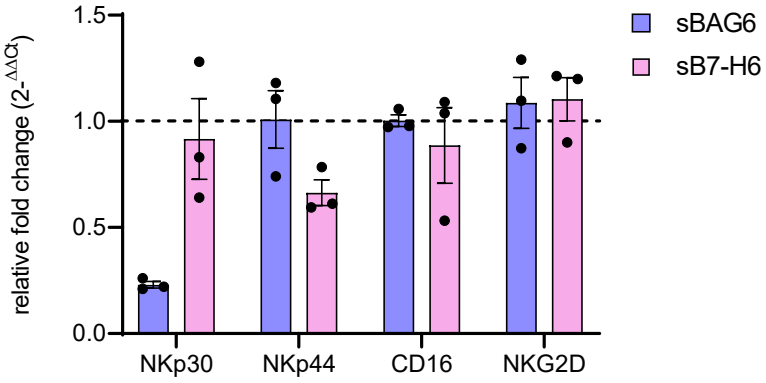
EV core facility

Soluble proteins alter the expression of NK cell receptors



Flow cytometry (NK cell panel)



RT-qPCR

sBAG6 affects NKp30 expression on the transcriptional level

Ponath et al. 2021

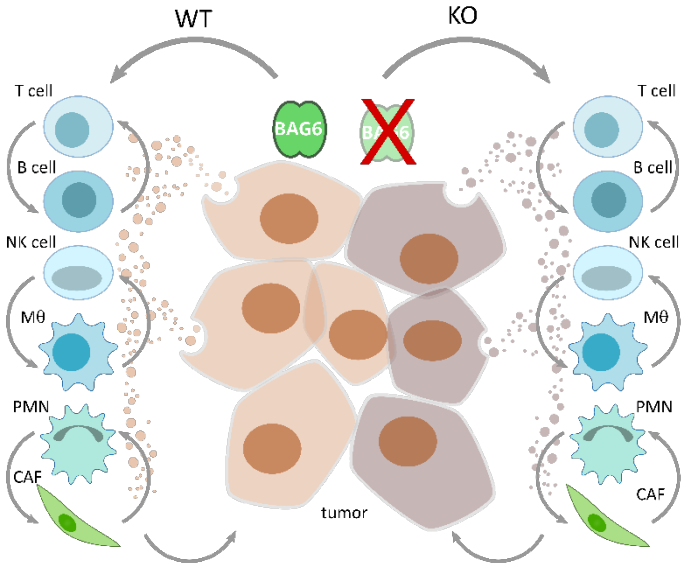
Aims and hypothesis

Hypothesis:

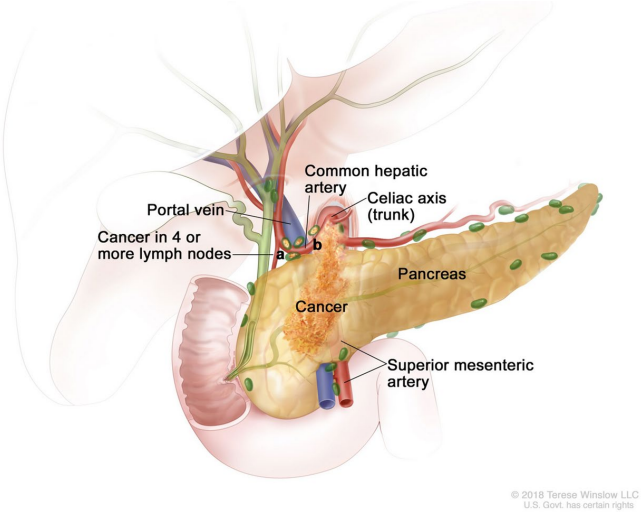
- BAG6 shapes the TME via immunoregulatory networks generated between the tumor and recruited immune cells
- BAG6 effects mediated by extracellular vesicles

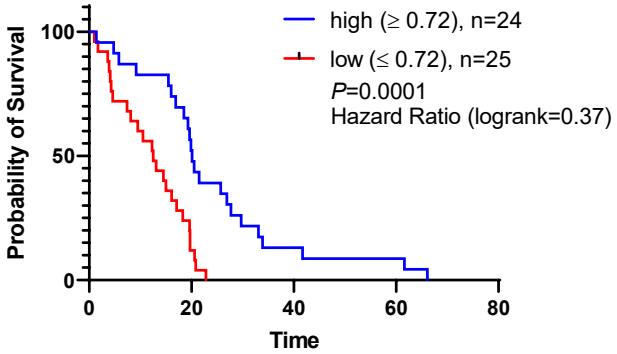
Aims:

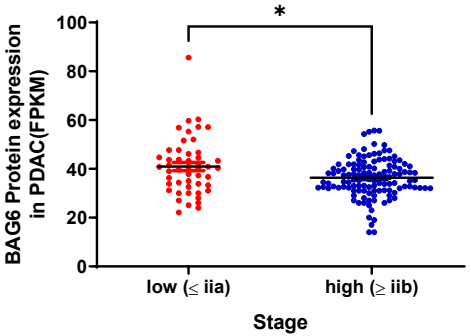
- Phenotyping of the tumor and recruited immune cells in the absence of BAG6.
- Proteomics and transcriptomics of tumor-EVs in WT and BAG6 KO.
- ***In vivo* study using *Cre-loxP and* single-cell sequencing to elucidate tumor-EVs' impact on the recruited immune cells.**



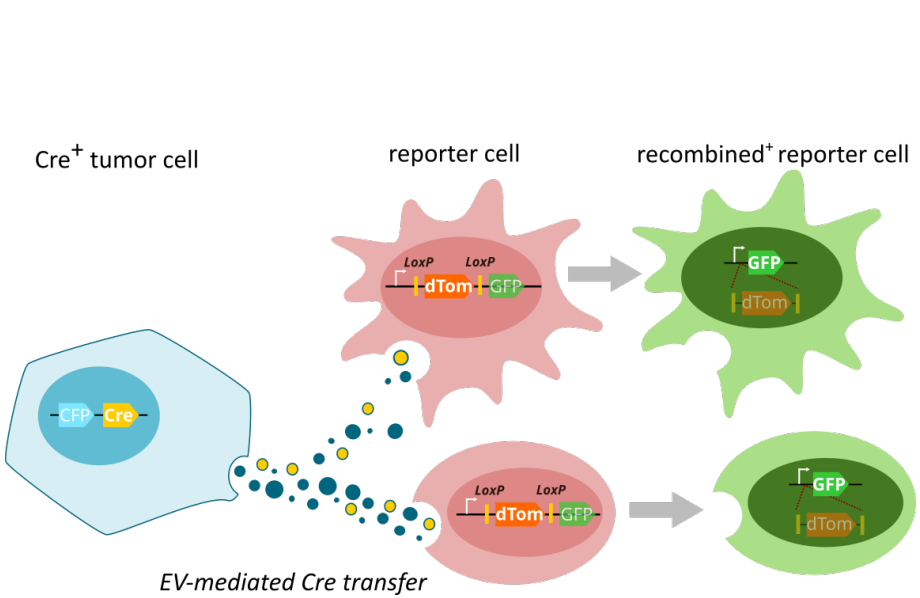
Low BAG6 expression level in pancreatic cancer correlates with a poor prognosis

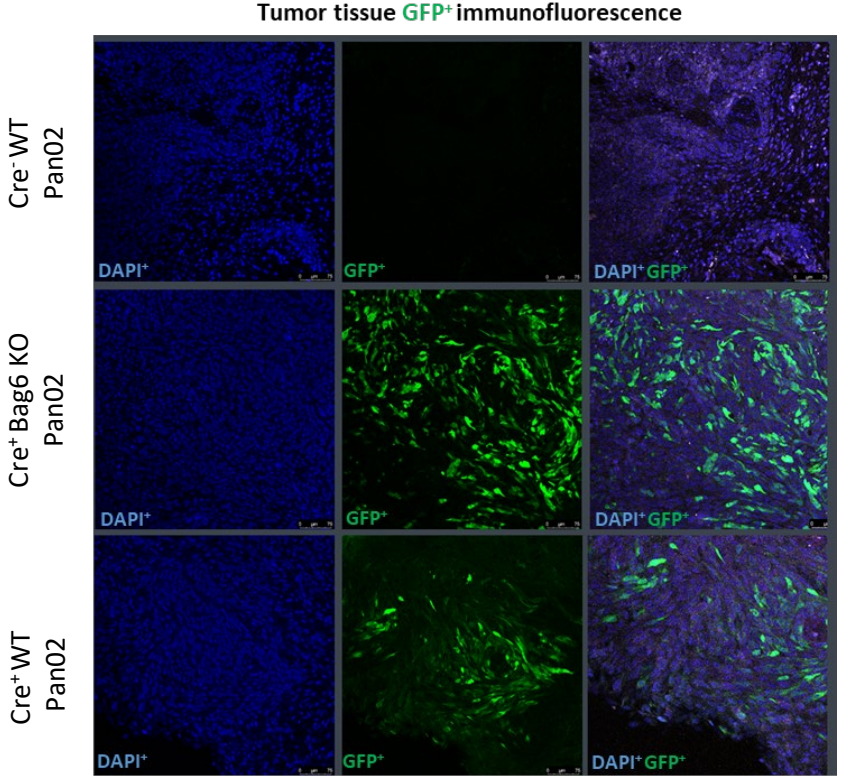






Using the Cre-loxP system for studying extracellular vesicle transfer





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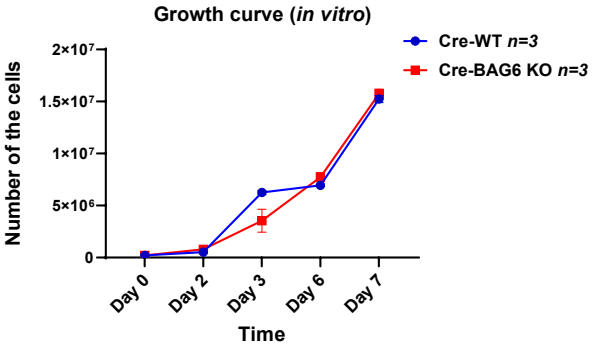
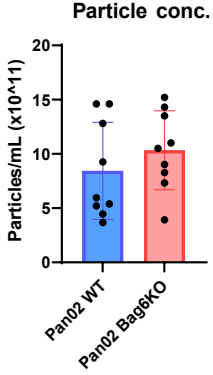
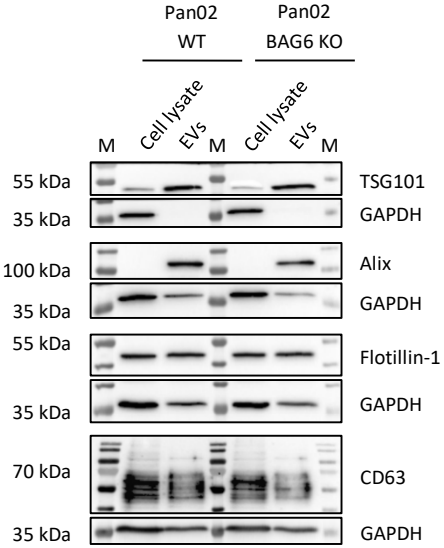
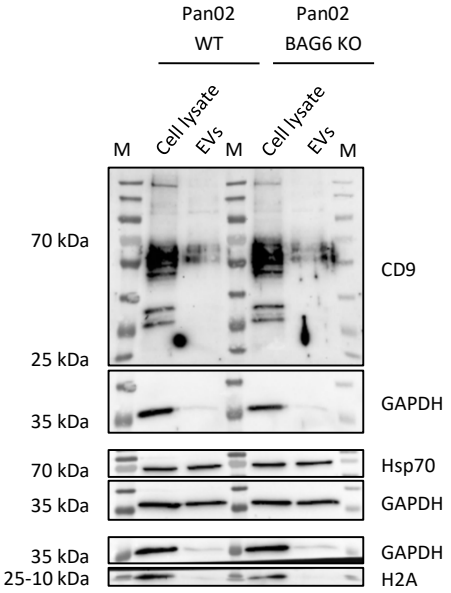




Nano-flow cytometry



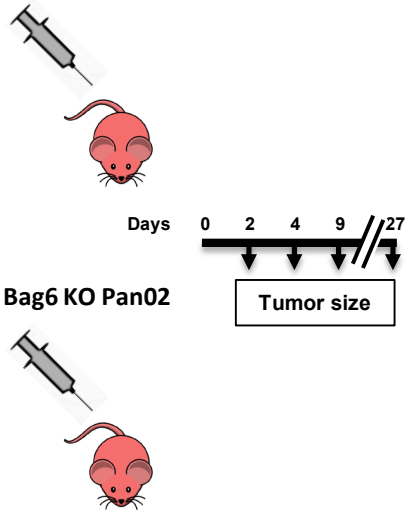


Western blot

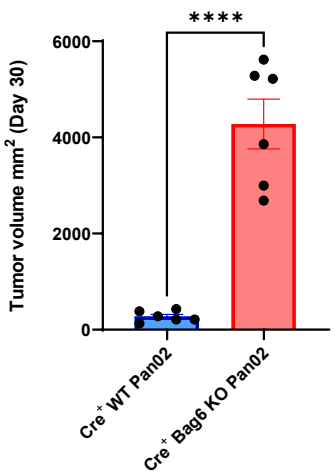
BAG6 impairs tumor growth in the preclinical pancreatic tumor mouse model

Subcutaneous model

Cre+ WT Pan02

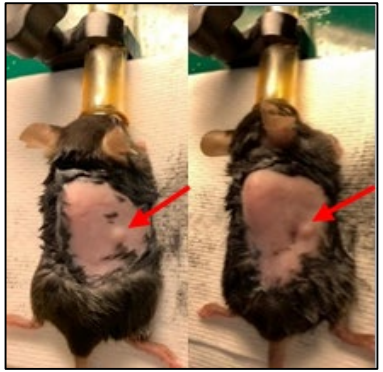


Cre+ Bag6 KO Pan02



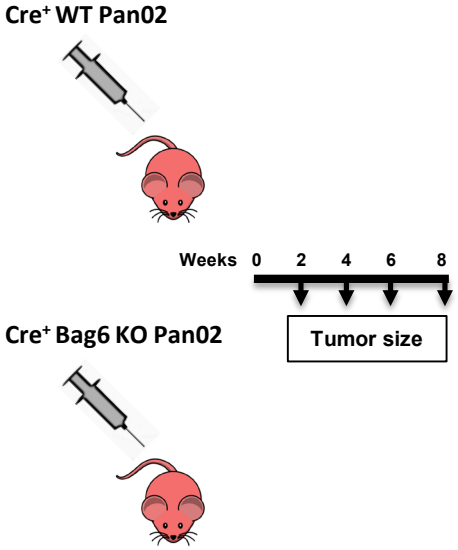
Cre+ Bag6 KO Pan02

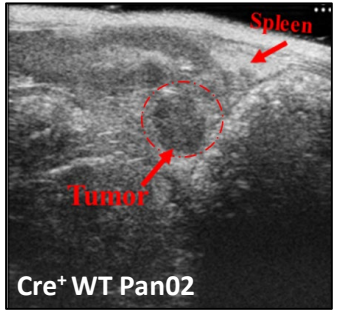
Cre+ WT Pan02

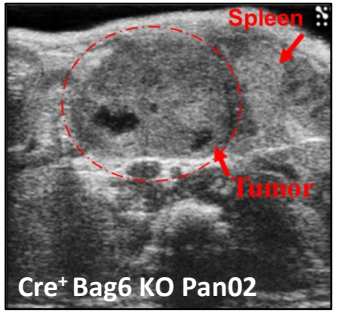


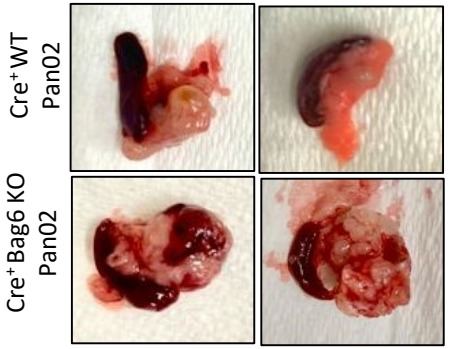
BAG6 impairs tumor growth in the preclinical pancreatic tumor mouse model

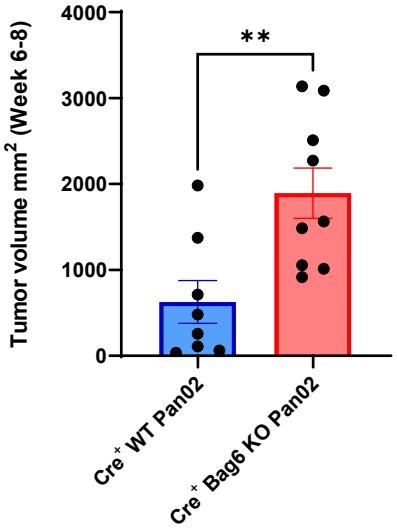
Orthotopic model



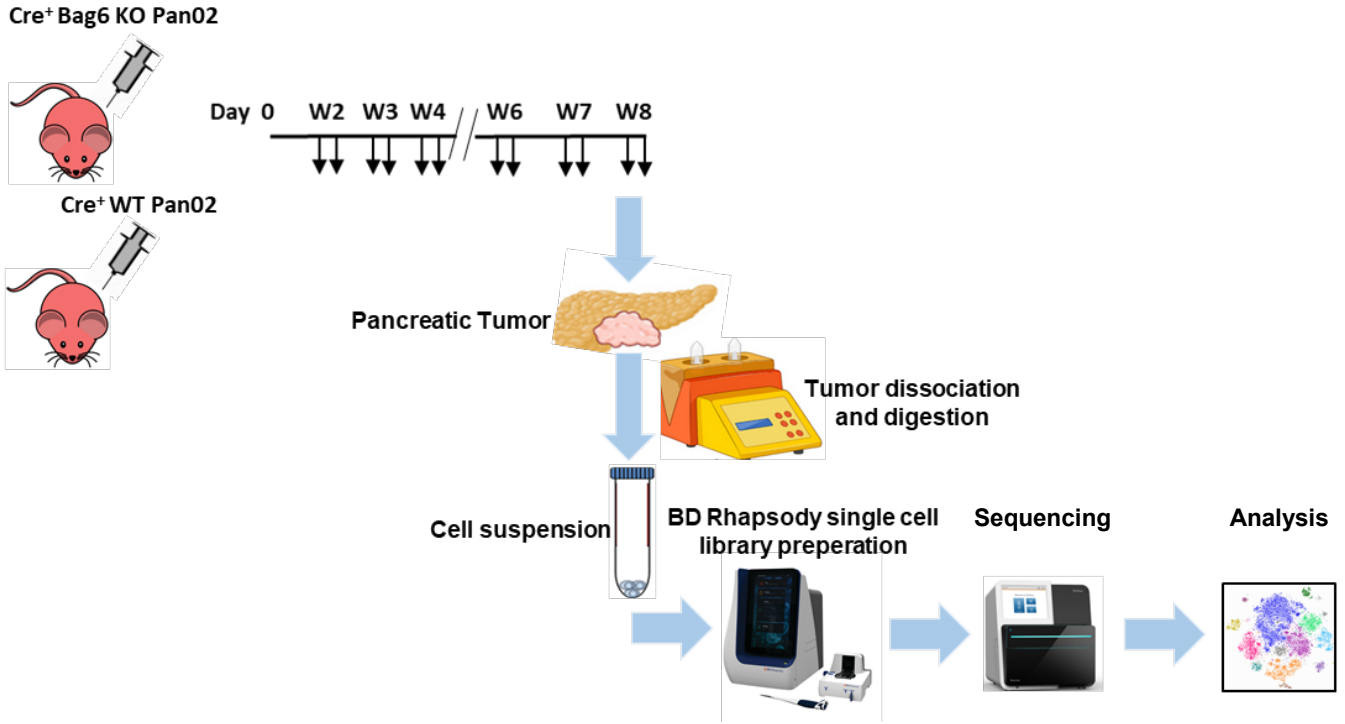








Combining single-cell sequencing and cre-loxP reporter for EV tracking



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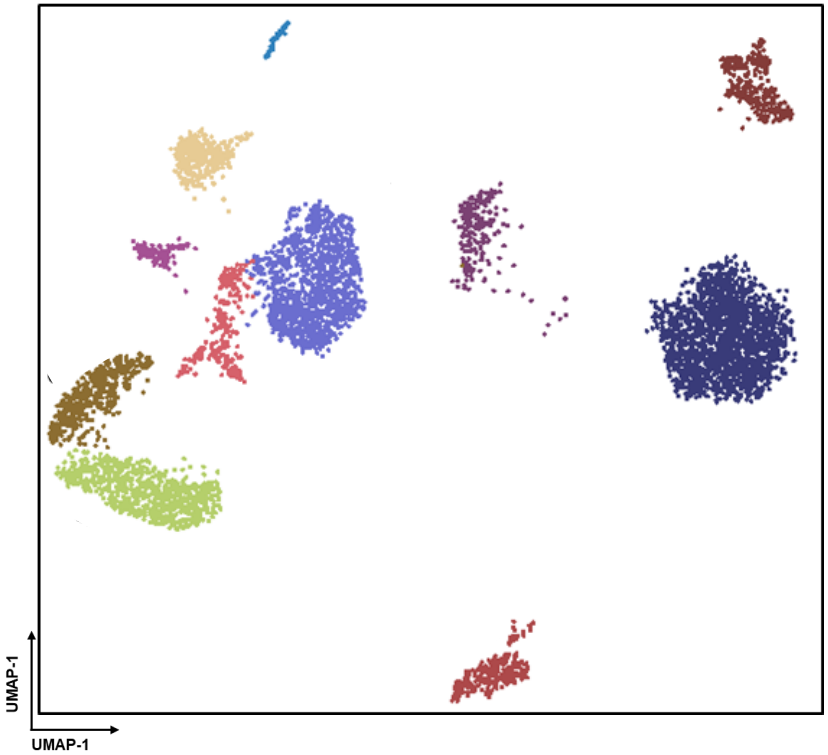

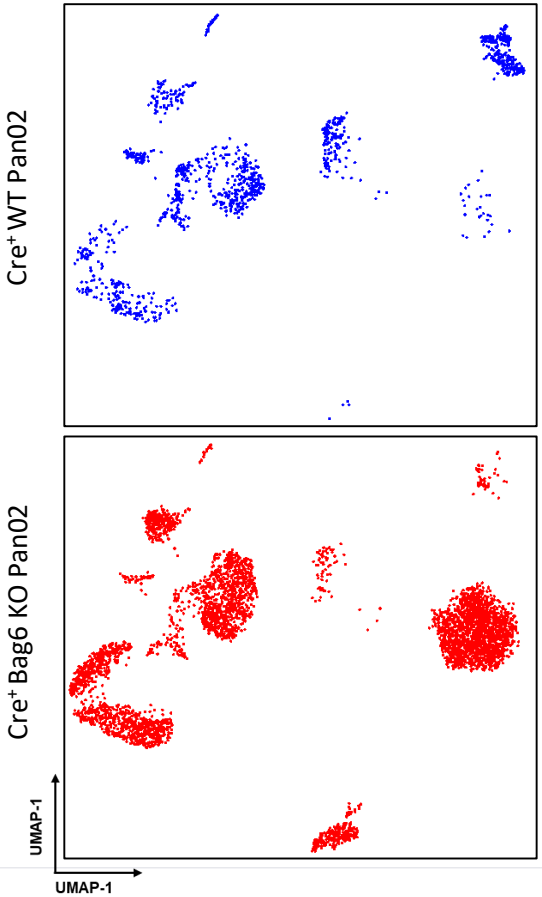

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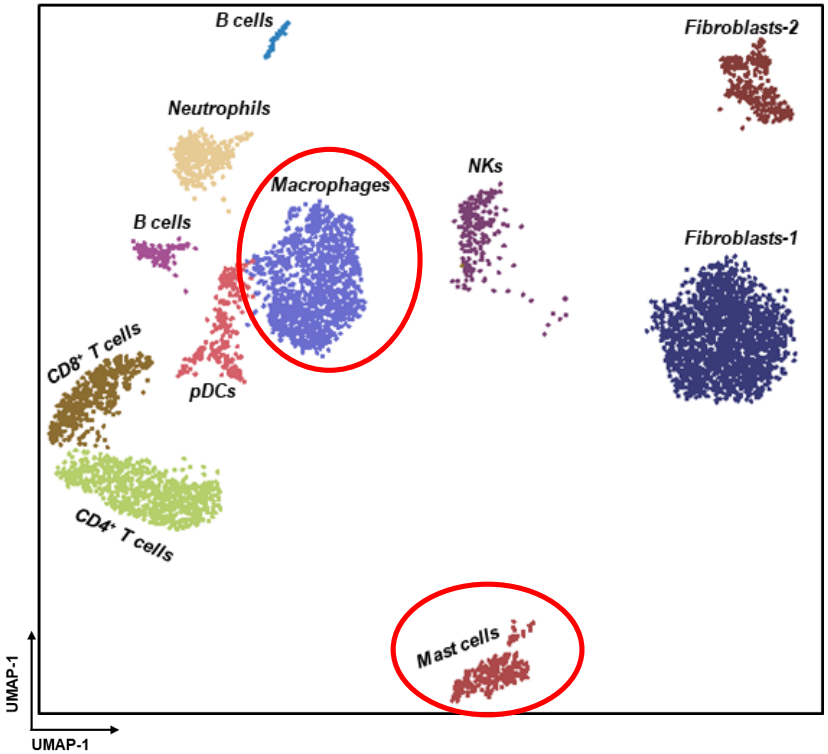

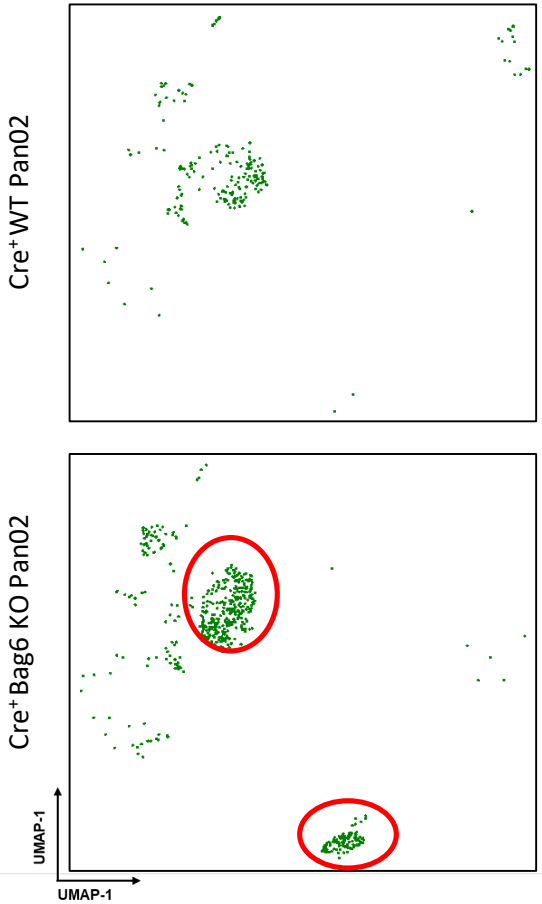

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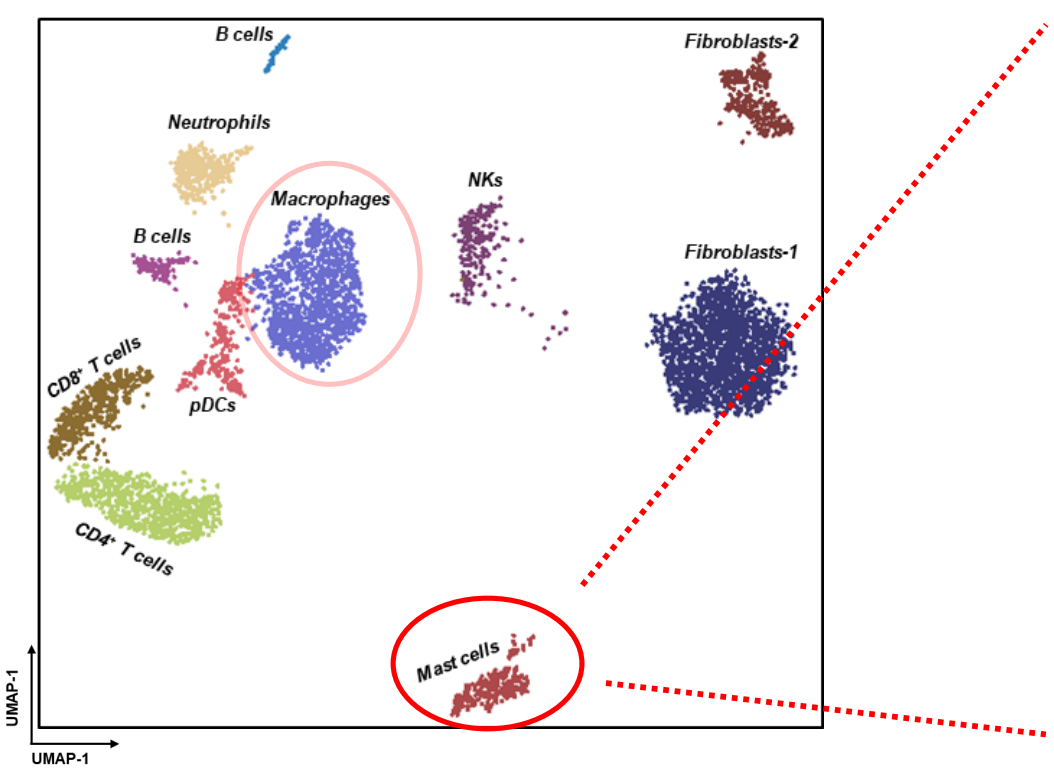

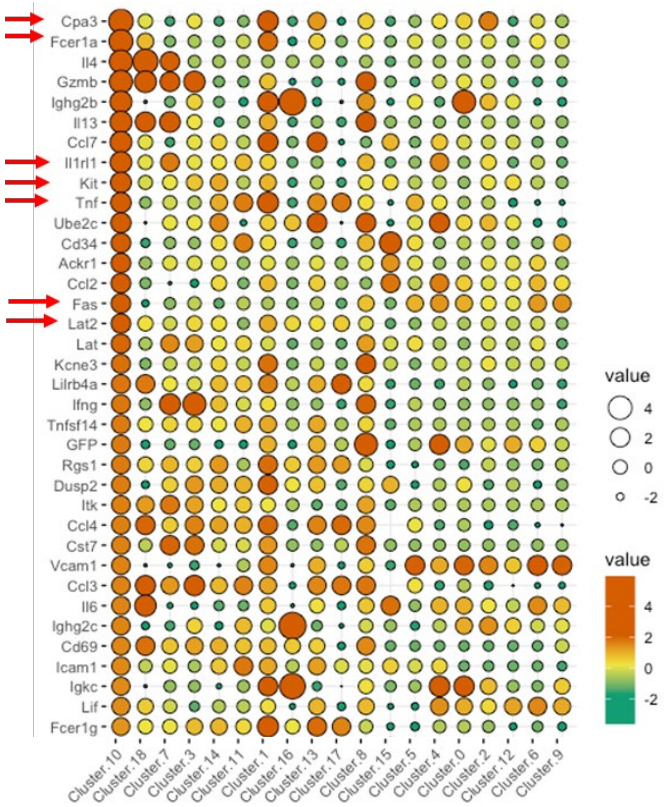

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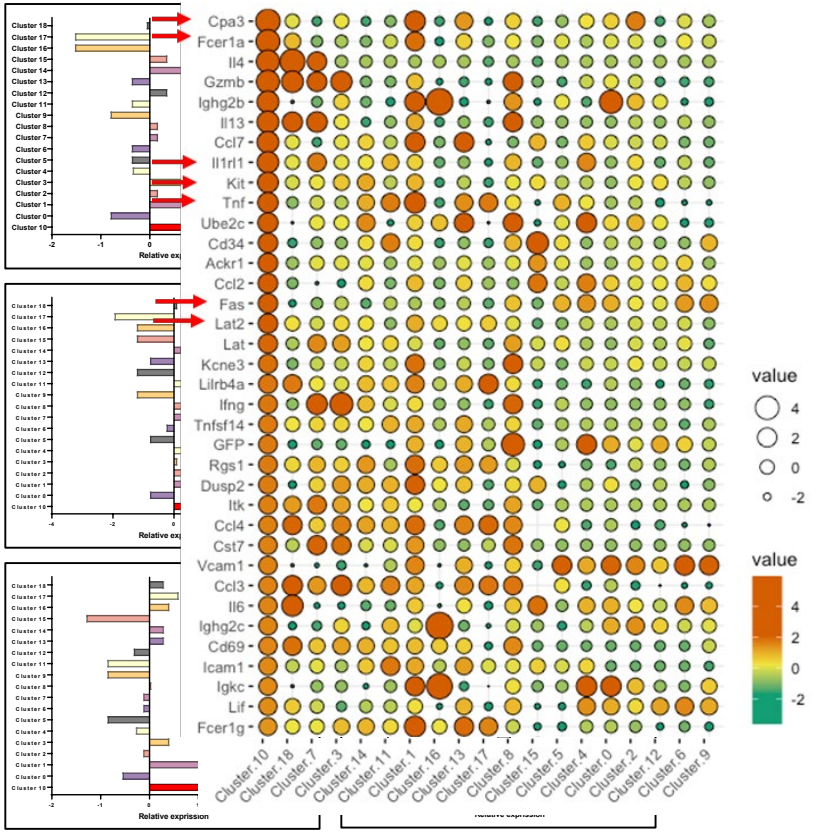

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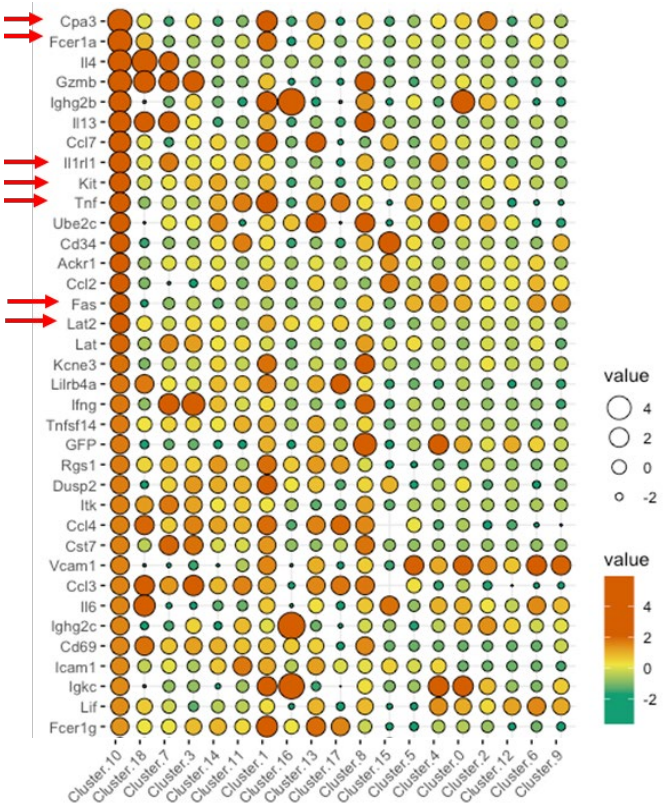

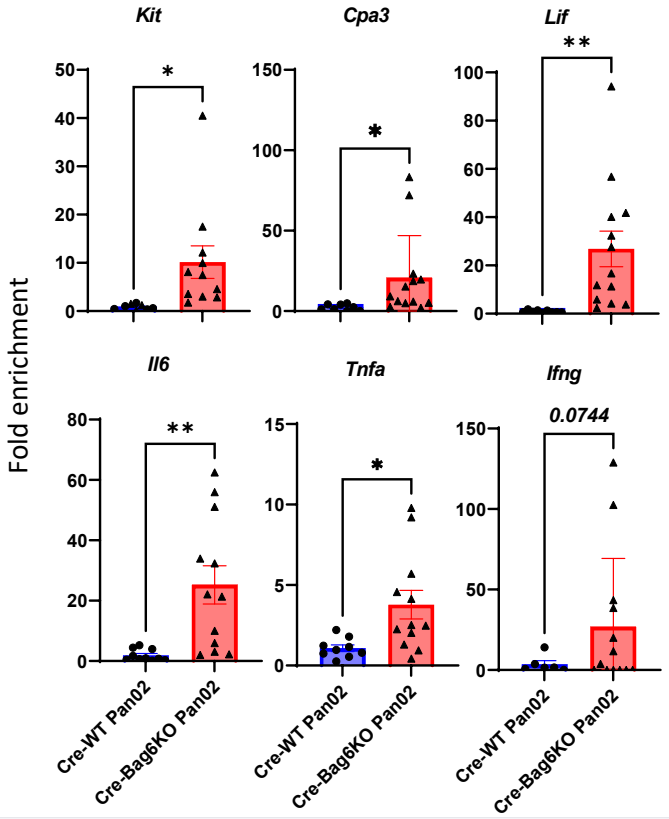

Summary

- Absence of BAG6 influences the tumor volume and aggressiveness in the preclinical model of pancreatic cancer.
- *Cre-loxP* approach in combination with single-cell sequencing as a novel method to study the impact of tumor-derived EVs on the phenotype of recipient cells in the TME.
- The absence of BAG6 is associated with enhanced EV uptake of tumor-recruited Mast cells, Macrophages, DCs, and Neutrophils.

Acknowledgements

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Katrin Roth

Translational Oncology
Florian Finkernagel

Contact:

E-Mail: preusserc@staff.uni-marburg.de

@ev_facility

DFG Deutsche Forschungsgemeinschaft

GRK 2573

GSEV German Society for Extracellular Vesicles

https://www.extracellular-vesicles.de/

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EV core facility

The EV future is bright!